Removing co-cultured T and NK like T cells by CD3-depletion during the expansion phase was possible and resulted in a higher purity of NK cells (56 - 96%). The cells retained their proliferative capacity after depletion and expansion factors of 30-156-fold were obtained.

Flow cytometry analysis revealed comparable expression of CD16, NKp30, NKp46 and NKG2D for manually or automatically expanded NK cells either from CD3-depleted or undepleted cultures.

Results show that large scale automation of NK cell expansion is possible by use of the CliniMACS® Prodigy. CD3 depletion of unwanted T and NK like T cells during culture is feasible.

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Human Di-Chimeric Cells a New Approach for Tolerance Inducing Protocols in Transplantation: A Preliminary Study

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Background: Successful vascularized composite allograft (VCA) transplantation requires life-long treatment combining more than two immunosuppressive agents and pose significant risks and side effects. Cell based therapies are a new promising approach for tolerance induction that could prevent or reduce negative impact of life-long immunosuppression. Bone marrow transplantation has already been tested for solid organs, face and upper extremity transplants for modulation of immune responses. We propose a new cellular therapy based on the ex vivo created donor-recipient chimeric cells as an alternative approach to bone marrow based therapies in support of VCA. The aim of this preclinical study was to create and characterize in vitro the phenotype, genotype and viability of fused human di-chimeric cells (dCC).

Materials and Methods: Fourteen ex vivo fusions of human umbilical cord blood (UCB) cells were performed. Mononuclear cells (MNCs) were isolated from UCB originating from 2 different donors. Next MNCs were stained separately by PKH26 and PKH67. Fusion procedure was performed using polyethylene glycol (PEG) technique. Double PKH26 and PKH67 stained cells were sorted out and subjected to further assessments. Flow cytometry (FC), (CD3, CD4, CD8, CD19, CD34 and CD90, viability test), confocal microscopy (CM), fluorescent lymphocytotoxicity assay (LCT), PCR-reverse sequence-specific oligonucleotide probe (PCR-rSSOP) and short tandem repeat- PCR (STR-PCR) and colony- forming unit (CFU) assay were assessed to characterize the phenotype and genotype of fused human chimeric cells.

Results: FC and CM analysis confirmed UCB fusion and creation of human dCC. Using LCT assay we determined that human dCC are sharing HLA class I and class II antigens specific for both types of UCB donors used for fusion. Results of the LCT test were confirmed by rSSOP and STR assay which revealed that fused dCC were in fact originating (39-51%) from each of the UCB donors. After fusion 96-99% of cells were viable. Preliminary phenotype characterization showed expression of all assessed markers on the surface of dCC. CFU assay confirmed the presence and functionality of dCC mature progenitor comparable to untreated cord blood progenitor cells.

Conclusions: We successfully confirmed feasibility of ex vivo fusion of UBC cells leading to creation of human fused dCC. We characterized cell phenotype and viability. This unique concept of di-chimeric cell therapy introduces new applications in transplant surgery. The ultimate goal is to induce tolerance in VCA transplants.

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Multi-Center Phase I Study of Th1/Tc1 Immunotherapy Following Autologous Hematopoietic Progenitor Cell Transplantation in Reccurrent or High Risk Plasma Cell Myeloma

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To date, despite recent progress in the treatment of Plasma Cell Myeloma (PCM), it remains an incurable disease in which prolonged therapy (maintenance) appears to be beneficial. Th1 immunity is critical in tumor immune surveillance and immune mediated tumor eradication, in particular of PCM. Immune reconstitution deficiency following autologous stem cell transplantation (AHCT) is believed to be a barrier to tumor eradication. Immunotherapy efforts and attempts to boost immunity with adoptive cell transfer have been thwarted by the short life span of the transferred cells. In our pre-clinical work, we found that human Th1/Tc1 lymphocytes, generated *ex vivo* in the presence of rapamycin ("T1.R"), prolonged engraftment in a human-into-murine model of xenogeneic GVHD. Based on these data, we have implemented a phase I, dose escalation clinical trial to evaluate the feasibility and toxicity of the adoptive transfer of autologous T1.R lymphocytes following AHCT in subjects with recurrent or newly diagnosed high-risk PCM. Subjects are receiving T1.R cells at doses ranging from $1x10^5$ to $45x10^6$ cells/kg. To generate T1.R cells, lymphocytes are collected by steady-state apheresis prior to hematopoietic progenitor cell mobilization/collection; elutriated lymphocytes are then cultured for 6 days using CD3, CD28 co-stimulation, polarizing cytokines (IL-2 + IFN-alpha), and high-dose rapamycin (1 μmol) and administered 6-8 weeks following AHCT. T1.R cells were generated in all cases (n=13); eight subjects have safely received T1.R cells at a median of 55 days post-AHCT. There have been no clinical adverse events attributable to the T1.R cells, and there has been no post-infusion serum elevation of the following cytokines tested at 1h, 4h, 24h, 48h, 72h and 7, 14 and 28 days: GM-CSF, INF-g, IL-1β, IL-6, IL-8 and TNF-α. Current accrual is proceeding at the 5 x 10^6 cells/kg cohort. T1.R cell recipients have in general shown a disease response (see Table), although the median post-